## TITLE OF INVENTION

## DNA IMMUNIZATION AGAINST *CHLAYMDIA* INFECTION

## FIELD OF INVENTION

The present invention relates to immunology and, in particular, to immunization of hosts using nucleic acid to provide protection against infection by *Chlaymdia*.

## REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of copending United States Patent Application No. 08/893,381 filed July 11, 1997.

## BACKGROUND OF THE INVENTION

DNA immunization is an approach for generating protective immunity against infectious diseases (ref. 1 - throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosure of these references are hereby incorporated by reference into the present disclosure). Unlike protein or peptide based subunit vaccines, DNA immunization provides protective immunity through expression of foreign proteins by host cells, thus allowing the presentation of antigen to the immune system in a manner more analogous to that which occurs during infection with viruses or intracellular pathogens (ref. 2). Although considerable interest has been generated by this technique, successful immunity has been most consistently induced by DNA immunization for viral diseases (ref. 3). Results have been more variable with non-viral pathogens which may reflect differences in the nature of the pathogens, in the immunizing antigens chosen, and in the routes of immunization (ref. 4). Further development of DNA vaccination will depend on elucidating the underlying immunological mechanisms and broadening its application

to other infectious diseases for which existing strategies of vaccine development have failed.

*Chlamydia trachomatis* is an obligate intracellular bacterial pathogen which usually remains localized to mucosal epithelial surfaces of the human host. Chlamydiae are dimorphic bacteria with an extracellular spore-like transmission cell termed the elementary body (EB) and an intracellular replicative cell termed the reticulate body (ref. 5). From a public health perspective, chlamydial infections are of great importance because they are significant causes of infertility, blindness and are a prevalent co-factor facilitating the transmission of human immunodeficiency virus type 1 (ref. 6). Protective immunity to *C. trachomatis* is effected through cytokines released by Th1-like CD 4 lymphocyte responses and by local antibody in mucosal secretions and is believed to be primarily directed to the major outer membrane protein (MOMP), which is quantitatively the dominant surface protein on the chlamydial bacterial cell and has a molecular mass of about 40 kDa (ref. 19).

Initial efforts in developing a chlamydial vaccine were based on parenteral immunization with the whole bacterial cell. Although this approach met with success in human trials, it was limited because protection was short-lived, partial and vaccination may exacerbate disease during subsequent infection episodes possibly due to pathological reactions to certain chlamydial antigens (ref. 8). More recent attempts at chlamydial vaccine design have been based on a subunit design using MOMP protein or peptides. These subunit vaccines have also generally failed, perhaps because the immunogens do not induce protective cellular and humoral immune responses recalled by native epitopes on the organism (ref. 9).

EP 192033 describes the provision of DNA construct for the expression, *in vitro*, of *Chlamydia trachomatis* MOMP polypeptides comprising the following operably linked elements:

a transcriptional promoter,

a DNA molecule encoding a *C. trachomatis* MOMP polypeptide comprising a MOMP polynucleotide at least 27 base pairs in length from a sequence provided in Appendix A thereto, and

a transcriptional terminator, wherein at least one of the transcriptional regulatory elements is not derived from *Chlamydia trachomatis*. There is no disclosure or suggestion in this prior art to effect DNA immunization with any such constructs.

WO 94/26900 describes the provision of hybrid picornaviruses which express chlamydial epitopes from MOMP of *Chlamydia trachomatis* and which is capable of inducing antibodies immuno-reactive with at least three different *Chlamydia* serovars. The hybrid picornavirus preferably is a hybrid polio virus which is attenuated for human administration.

## SUMMARY OF THE INVENTION

The present invention is concerned with nucleic acid immunization, specifically DNA immunization, to generate in a host protective antibodies to a MOMP of a strain of *Chlamydia*. DNA immunization induces a broad spectrum of immune responses including Th1-like CD4 responses and mucosal immunity.

Accordingly, in one aspect, the present invention provides an immunogenic composition for *in vivo* administration to a host for the generation in the host of a protective immune response to a major outer membrane protein (MOMP) of a strain of *Chlamydia*, comprising a non-replicating vector comprising a nucleotide sequence encoding a MOMP or MOMP fragment

that generates a MOMP-specific immune response, and a promoter sequence operatively coupled to the nucleotide sequence for expression of the MOMP or MOMP fragment in the host; and a pharmaceutically-acceptable carrier therefor.

The nucleotide sequence may encode a full-length MOMP protein or may encode a fragment, such as the N-terminal half of MOMP or a fragment that encompasses epitopic sequences. The nucleotide sequence may encode a MOMP or MOMP fragment which stimulates a recall immune response following exposure to wild-type *Chlamydia*. The promoter may be the cytomegalovirus promoter.

The fragment that encompasses epitopic sequences may include one or more conserved domain (CD) sequences and/or one or more variable domain (VD) sequences of MOMP from a strain of *Chlamydia*. In particular, the fragment may encompass the CD2 and VD2 sequences, CD3 and VD3 sequences and CD5 sequence. Clones containing nucleotide sequences encoding such fragments are termed clones CV2, CV3 and CD5 herein. Clones CV2 encompasses nucleotides 247 to 468 of *Chlamydia trachomatis* MOMP gene, clone CV3 encompasses nucleotides 469 to 696 of *Chlamydia trachomatis* MOMP gene and clone CV5 encompasses nucleotides 931 to 1098 of *Chlamydia trachomatis* MOMP gene. Non-replicating vectors comprising such sequences are novel and constitute further aspects of the invention.

Accordingly, in an additional aspect of the invention, there is provided a non-replicating vector, comprising a nucleotide sequence encoding a region comprising at least one of the conserved domains 2, 3 and 5 of a major outer membrane protein of a strain of *Chlamydia*, and a promoter sequence operatively coupled to the nucleotide sequence for expression of the at least one conserved domain in a host. In this aspect of

the invention, the various options and alternatives discussed above and below may be employed.

The strain of *Chlamydia* may be a strain of *Chlamydia* inducing chlamydial infection of the lung, including *Chlamydia trachomatis* or *Chlamydia pneumoniae*. The non-replicating vector may be plasmid pcDNA3 into which the nucleotide sequence is inserted. The immune response which is stimulated may be predominantly a cellular immune response.

In a further aspect of the invention, there is provided as a method of immunizing a host against disease caused by infection with a strain of *Chlamydia*, which comprises administering to the host an effective amount of a non-replicating vector comprising a nucleotide sequence encoding a major outer membrane protein (MOMP) of a strain of *Chlamydia* or a MOMP fragment that generates a MOMP-specific immune response, and a promoter sequence operatively coupled to the nucleotide sequence for expression of the MOMP or MOMP fragment in the host.

In this aspect of the present invention, the various options and alternatives discussed above may be employed.

The non-replicating vector may be administrated to the host, including a human host, in any convenient manner, such as intramuscularly or intranasally. Intranasal administration stimulated the strongest immune response in experiments conducted herein.

The present invention also includes, in an additional aspect thereof, a method of using a gene encoding a major outer membrane protein (MOMP) of a strain of *Chlamydia* or MOMP fragment that generates a MOMP-specific immune response, to produce an immune response in a host, which comprises isolating the gene, operatively linking the gene to at least one control

sequence to produce a non-replicating vector, the control sequence directing expression of the MOMP or MOMP fragment when introduced into a host to produce an immune response to the MOMP or MOMP fragment, and introducing the vector into a host.

A further aspect of the present invention provides a method of producing a vaccine for protection of a host against disease caused by infection with a strain of *Chlamydia*, which comprises isolating a nucleotide sequence encoding a major outer membrane protein (MOMP) of a strain of *Chlamydia* or a MOMP fragment that generates a MOMP-specific immune response, operatively linking the nucleotide sequence to at least one control sequence to produce a non-replicating vector, the control sequence directing expression of the MOMP or MOMP fragment when introduced to a host to produce an immune response to the MOMP or MOMP fragment, and formulating the vector as a vaccine for *in vivo* administration to a host. The invention extends to the vaccine produced by this method.

Advantages of the present invention, therefore, include a method of obtaining a protective immune response to infection carried by a strain of *Chlamydia* by nucleic acid immunization of nuelcic acid sequence encoding the major outer membrane protein of a strain of *Chlamydia* or a fragment of the outer membrane protein that generates a MOMP-specific immune response.

**BRIEF DESCRIPTION OF DRAWINGS**

Figure 1 illustrates delayed-type hypersensitively (DTH) responses in mice following immunization. Balb/c mice (four per group) were immunized intramuscularly (pMOMP IM) or intranasally (pMOMP IN) with plasmid DNA containing the coding sequence of the MoPn MOMP gene or with MoPn elementary bodies (EB) at 0,3,6 weeks. The control group was treated with the blank plasmid vector

(pcDNA3). Fifteen days after the last immunization, mice were tested for MoPn-specific DTH response as follows: 25 μl of heat-inactivated MoPn EB ($5 \times 10^4$ IFU) in SPG buffer was injected into the right hind footpad and the same volume of SPG buffer was injected into the left hind footpad. Footpad swelling was measured at 48H and 72H following the injection. The difference between the thickness of the two footpads was used as a measure of the DTH response. Data are shown in Figure 1 as the mean ± SEM.

Figure 2, comprising panels A and B, illustrates protection against MoPn infection with *momp* gene products following DNA immunization. Balb/c mice were immunized with (o) pcDNA3 (n = 11), (•) pMOMP intramuscularly (n = 12), (Δ) pMOMP intranasally (n = 5) or (▲) MoPn EBs (n = 12). Eighteen days after the last immunization, mice were challenged intranasally with infectious MoPn (1000 IFU). Panel A shows body weight loss. Body weight was measured daily following infection challenge and each point in Figure 2, panel A, represents the mean ± SEM of the body weight loss. Panel B shows *in vivo* chlamydia clearance. Mice were sacrifced day 10 postinfection and recovery of infectious MoPn from lung tissue was analyzed by quantitative tissue culture in order to deterine the *in vivo* chlamydial clearance. The data in Figure 2B, panel B, represent mean ± SEM of the $\log_{10}$ IFU per lung.

Figure 3 illustrates detection of serum antibody to MoPn MOMP in DNA immunized mice by immunoblot analysis. Day 60 pooled sera from mice immunized with MoPn EBs (Lane A), pMOMP (Lane B), blank pcDNA3 vector (Lane C) or saline (Lane D), were diluted at 1:100 and reacted with purified MoPn EBs that had been separated in a 10%

SDS-polyacrylamide gel and transferred to a nitrocellulose membrane.

Figure 4, comprising panels A, B, C and D, compares serum 1gG subclasses $1gG_{2a}$ (Panels A and C) with 1gG, Panels B and D) against recombinant MOMP protein (Panels A and B) or MoPn EBs (Panels C and D) induced by DNA immunization. Mice were non-immunized or immunized intramuscularly with pMOMP, CTP synthetase DNA (pCTP) or the blank plasmid vector (pcDNA3) at 0,3,6 weeks and pooled sera from each group were collected two weeks following the last immunization (day 10). The data in Figure 4 represent mean ± SEM of the OD value of four duplicates.

Figure 5, comprising panels A and B, demonstrates that DNA vaccination with the MOMP gene enhanced clearance of MoPn infection in the lung. Groups of Balb/c mice were immunized with pMOMP (n = 10), pcDNA3 (n = 10) or saline (n = 5). Eighteen days after the last immunization, the mice were challenged intranasally with infectious MoPn ($10^4$ IFU). Panel A shows the body weight of the mice measured daily following challenge infection until the mice were sacrificed at day 10. Each point in Figure 5, panel A, represents the mean ± SEM of the body weight change. * represents $\underline{P} < .05$ compared with pcDNA3 treated group. Panel B: the mice were sacrificed at day 10 postinfection and the MoPn growth in the lung was analyzed by quantitative tissue culture. The data in Figure 5, panel B, represent mean ± SEM of the $Log_{10}$IFU per lung. * represents $\underline{P} < .01$ compared with pcDNA3 treated group.

Figure 6, comprising panels A and B, shows evaluation of the responses of mice to MoPn intranasal challenge infection. Panel A shows the change in body weight post challenge and Panel B shows the growth of

MoPn in lung tissue collected 10 days after challenge. Mice were sham immunized, immunized intraperitoneally with MoPn EBs recovered from prior MoPn lung infection, or immunized intramuscularly with p½MOMP.

Figure 7 shows the elements and construction of plasmid pcDNA3/MOMP, 6495 bp in size.

Figure 8 shows schematically the nucleotide structure of the mature MOMP gene of *C. trachomatis* MoPn strain with conserved (CD) and variable (VD) domains identified as well as clones formed by cloning the identified sequences into pcDNA3, as described below in the Examples.

Figure 9 shows the loss in body weight (in grams) following intranasal challenge with 5 x $10^3$ IFU of MoPn among groups of Balb/c mice intramuscularly immunized with blank vector (pcDNA3), with pcDNA3 into which is individually cloned CV1 to CD5 encoding MOMP nucleotide sequences (CV1 etc), and with pcDNA3 into which the whole MOMP encoding nucleotide sequence is cloned (pMOMP).

Figure 10 shows the results of assays to determine growth of *C. trachomatis* on day 10 in lungs of mice challenged with 5 x $10^3$ IFU of MoPn following intramuscular immunization with blank vector (pcDNA3), with pcDNA3 into which is individually cloned CV1 to CD5 encoding MOMP nucleotide sequences (pCV1 etc), and with pcDNA3 into which the whole MOMP encoding nucleotide sequence is cloned (pMOMP).

Figure 11 shows footpad swelling reactions (DTH) 48 hours after footpad injection of 2 x $10^5$ IFU of inactivated MoPn EBs among groups of Balb/c mice intramuscularly immunized with blank pcDNA3 vector (PC), with pcDNA3 into which is individually cloned CV1 to CD5 encoding MOMP nucleotide sequences (CV1 etc), and with

pcDNA3 into which the whole MOMP encoding nucleotide sequence is cloned (pM).

Figure 12 shows the proliferation responses of splenocytes at day 60 post immunization after *in vitro* stimulation with whole inactivated MoPn EBs for 96 hours among groups of Balb/c mice immunized with blank pcDNA3 vector (pc), with pcDNA3 into which is individually cloned CV1 to CD5 encoding MOMP nucleotide sequences (CV1 etc), and with pcDNA3 into which the whole MOMP encoding nucleotide sequences is cloned (pM).

Figure 13 shows the poliferation responses of splenocytes to the same constructs is in Figure 11, except that the results are expressed as a stimulation index (SI).

Figure 14 shows the interferon-γ secretion response of MoPn stimulated splenocytes collected on day 60 after immunization among groups of Balb/c mice immunized with blank pcDNA3 vector (pc), with pcDNA3 into which is individually cloned CV1 to CD5 encoding MOMP nucleotide sequences (CV1 etc), and with pcDNA3 into which the whole MoPn MOMP encoding nucleotide sequence is cloned (pM).

Figure 15 shows the IgG2a antibody titer to whole MoPn EBs using sera collected at day 60 after immunization among groups of Balb/c mice immunized with blank pcDNA3 vector (pc), with pcDNA3 into which is individually cloned CV1 to CD5 encoding MOMP nucleotide sequences (CV1 etc), and with pcDNA3 into which the whole MOMP encoding nucleotide sequences is cloned (pM).

Figure 16 shows the IgG2a antibody titer to whole MoPn EBs using sera collected at day 60 after intramuscularly immunizing groups of Balb/c mice with blank pcDNA3 vector (pc), pcDNA3 containing the whole MoPn encoding nucleotide sequence (pM), and with pcDNA3

containing the whole serovar C MOMP encoding nucleotide sequence (pM(C)).

Figure 17 shows the 48 hour footpad swelling responses (DTH) to injection with 2 x $10^5$ IFU whole inactivated MoPn EBs among groups of Balb/c mice intramuscularly immunized 60 days previously with empty plasmid pcDNA3 vector (pc), pcDNA3 containing the whole MoPn encoding nucleotide sequence (pM), and with pcDNA3 containing the whole serovar C MOMP encoding nucleotide sequence (pM(C)).

Figure 18 shows the 96 hour proliferation of MoPn EB simulated splenocytes, expressed as a stimulation index (SI), collected from groups of Balb/c mice intramuscularly immunized with empty plasmid pcDNA3 vector (pc), pcDNA3 containing the whole MoPn MOMP encoding nucleotide sequence (pM), and with pcDNA3 containing the whole serovar C encoding nucleotide sequence (pM(C)) sixty days previously.

Figure 19 shows the IFN-γ secretion of MoPn EBs stimulated splenocytes collected from groups of Balb/c mice intramuscularly immunized 60 days previously with empty pcDNA3 plasmid (pc), pcDNA3 containing the whole MoPn MOMP encoding nucleotide sequence (pM), and with pcDNA3 containing the whole serovar C encoding nucleotide sequence (pM(C)).

Figure 20 shows a comparison of the amino acid sequence of MOMP sequences (SEQ ID NOS: 1 to 15) from a variety of serovars of *C. trachomatis*. Residues which are identical to serovar E MOMP are represented by dots. The four VDs (VDI to VDIV) and the conserved cysteines are boxed by solid line. The conserved position where one cysteine is located in all *C. trachomatis* and *C. pneumonitis* MOMP sequences, but where one serine is located in GPIC and Mn MOMPs, is boxed by a broken line.

Numbers above boxes denote amino acid residues of serovar E MOMP only.

GENERAL DESCRIPTION OF THE INVENTION

To illustrate the present invention, plasmid DNA was constructed containing the MOMP gene and MOMP gene fragments from the *C. trachomatis* mouse pneumonitis strain (MoPn), which is a natural murine pathogen, permitting experimentation to be effected in mice. It is known that primary infection in the model induces strong protective immunity to reinfection. For human immunization, a human pathogen strain is used, such as serovar C of *C. trachomatis*.

Any convenient plasmid vector may be used for the MOMP gene or fragment, such as pcDNA3, a eukaryotic II-selectable expression vector (Invitrogen, San Diego, CA, USA), containing a cyotmegalovirus promoter. The MOMP gene or MOMP gene fragment may be inserted in the vector in any convenient manner. The gene or gene fragments may be amplified from *Chlamydia trachomatic* genomic DNA by PCR using suitable primers and the PCR product cloned into the vector. The MOMP gene-carrying plasmid may be transferred, such as by electroporation, into *E. coli* for replication therein. A MOMP-carrying plasmid, pcDNA3/MOMP, of 6495 bp in size, is shown in Figure 7. Plasmids may be extracted from the *E. coli* in any convenient manner.

The plasmid containing the MOMP gene or MOMP gene fragment may be administered in any convenient manner to the host, such as intramuscularly or intranasally, in conjunction with a pharmaceutically-acceptable carrier. In the experimentation outlined below, it was found that intranasal administration of the plasmid DNA elicited the strongest immune response.

The data presented herein and described in detail below demonstrates that DNA immunization with the *C.*

*trachomatis* MOMP gene and MOMP gene fragments elicits both cellular and humoral immune responses and produces significant protective immunity to lung challenge infection with *C. trachomatis* MoPn. The results are more encouraging than those obtained using recombinant MOMP protein or synthetic peptides as the immunogen and suggest that DNA immunization is an alternative method to deliver a chlamydial subunit immunogen in order to elicit the requisite protective cellular and humoral immune responses.

The data presented herein also demonstrate the importance of selection of an antigen gene or gene fragment for DNA immunization. The antigen gene elicits immune responses that are capable of stimulating recall immunity following exposure to the natural pathogen. In particular, injection of a DNA expression vector encoding the major surface protein (pMOMP) or fragment thereof but not one encoding a cytoplasmic enzyme (CTP synthetase) of *C. trachomatis*, generated significant protective immunity to subsequent chlamydial challenge. The protective immune response appeared to be predominantly mediated by cellular immunity and not by humoral immunity since antibodies elicited by DNA vaccination did not bind to native EBs. In addition, MOMP DNA but not CTP synthetase DNA immunization elicited cellular immunity readily recalled by native EBs as shown by positive DTH reactions.

In addition, mucosal delivery of MOMP DNA is demonstrated herein to be significantly more efficient in inducing protective immunity to *C. trachomatis* infection than intramuscular injection. This may be relevant to the nature of *C. trachomatis* infection which is essentially restricted to mucosal surfaces and the efficiency of antigen presentation (ref. 14). The rich population and rapid recruitment of dendritic cells into

the respiratory epithelium of the lung may be relevant to the enhanced efficacy of intranasal DNA immunization experiments (ref. 15). The data presented herein represents the demonstration of a first subunit chlamydial vaccine which engenders substantial protective immunity.

Additionally, it may be possible to amplify (and/or canalize) the protective immune response by co-administration of DNAs that express immunoregulatory cytokines in addition to the antigen gene in order to achieve complete immunity (ref. 21) The use of multiple antigen genes from chlamydiae may augment the level of protective immunity achieved by DNA vaccination.

A possible concern regarding MOMP DNA immunization stems from the observation that the MOMP among human *C. trachomatis* strains is highly polymorphic (ref. 16) and hence it may be difficult to generate a universal chlamydial vaccine based on this antigen gene. One way to solve this problem may be to search for conserved protective epitope(s) within the MOMP molecule. As seen in the results presented below, certain vectors containing nucleotide sequences encoding conserved and variable domains, identified in Figure 8, or conserved domains generated a protective immune response, as determined by loss of body weight, as shown in Figure 9. Figure 10 shows that the pCV3 and pCD5 immunogen evoked a protective immune response to MoPn challenge as measured by *in vivo* growth of MoPn in lung tissue day 10, with challenge and comparable to pMOMP.

Figures 12 and 13 show the proliferation responses of splenocytes to the vectors containing the conserved and variable domains and the whole MOMP gene. These responses were determined in the following manner. Mice were sacrificed two weeks after the fourth immunization. The spleens were removed and single-cell suspensions

were prepared. 200 μl of the cell suspension ($5 \times 10^5$ well) in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum (FCS), 1% L-glutamine and $5 \times 10^{-5}$ M 2-mercaptoethanol (2ME, Kodak, Rochester, NY) were incubated with $1 \times 10^5$ IFU/ml of MoPn in 96 well flat bottom plates in triplicate 37°C in 5% $CO_2$ for 96 hours. Negative control wells contained spleen cells without antigen and positive control wells contained spleen cells with 0.25 μg/ml of concanavalin A. 0.25 μCi/well of tritiated ($^3H$) thymidine (2 Ci/mmol, 74 Gbq/mmol, imCi/ml, ICN, Irvine, CA) was added after 3 days of culture and 16h before harvest. The cells were harvested with a PHD cell harvester (Cambridge Technology Inc., Watertown, MA, USA) and counted in 2ml of scintillation solution (Universal, ICN, Costa Mesa) in a Beckman LS5000 counter (Beckman Instrument, UK).

The results obtained are set forth in Figures 12 and 13, which show that pCV3 and pMOMP elicit a cell mediated immune response.

Figure 14, which shows interferon-γ secretion responses of the splenocytes, to the vectors containing the conserved and variable domains and the whole MOMP gene. These responses were determined in the following manner. A cytokine-specific ELISPOT assay was used for the quantification of murine IFNγ and IL-10 secreting cells in the murine spleen. For all assays 96-well nitrocellulose-based microtiters (Milititer Multiscreen HA plates, Millipore Corp, Molshem, France) were coated overnight at 4°C with 100 μl of the anti-cytokine mAb diluted in PBS at a concentration of 5 μg/ml. After removing the coating solution from the plates, wells were blocked for at least 1 hour with RPMI-1640 media containing 40% fetal calf serum at 37°C, in $CO_2$. After

rinsing the plates with PBS-T once, the testing cells were added into the wells.

For induction of antigen specific IFN$\gamma$ secreting cells in immunized mice, single cells were adjusted to 5 x $10^6$ cells/ml and cultured with 2 x $10^5$ IFU/ml of UV-killed EB of MoPn in 24 well plates for 72 hours. After washing with RPMI 1640, cells were added onto the 96-well plates for 72 hours. After washing with RPMI 1640, cells were added onto the 96-well nitrocellulose-based microtiter plates which had been previously coated with anti-cytokine antibodies. The cells were added to individual wells (2 x $10^5$ or 1 x $10^5$/100-$\mu$l/well) and incubated for 24 hours at 37°C in a $CO_2$ incubator. Wells were rinsed extensively with PBS-T containing 1% BSA. Following rinsing with PBS-T three times (removing the supporting manifold and washing the back of the plate thoroughly with PBS-T), alkaline phosphatase conjugated streptavidin in PBS containing 1% BSA at 1:2000 at a concentration of 0.5 $\mu$g/ml was added and incubated at 37°C in $CO_2$ for 45 min. After rinsing thoroughly, 100 $\mu$l/well of the colormetric substrate phosphate BICP (5-bromo-4-chloro-3-indolyl phosphate)/NBT (Nitro blue tetrazolium) at 0.16 mg/ml BICP and 1 mg/ml NBT in substrate buffer (0.1 M NaCl, 0.1M Tris, pH 9.5, 0.05 M $MgCl_2$) was added and incubated at room temperature until spots were visualized. The reaction was stopped by the addition of water.

The results obtained in Figure 14 suggest that cytokine generation may not necessarily be a correlate of a protective immune response.

Figure 15 shows $IgG_{2a}$ antibody titers in sera collected from the mice 60 days immunization by the vectors containing the conserved and variable domains and full length MOMP gene. Only in the case of

immunization by pCV3 and pCV5, was an $IgG_{2a}$ immune response generated, indicating that a Th1-like response was elicited by these vectors.

Another, possibly more feasible, way is to design a multivalent vaccine based on multiple MOMP genes. The latter approach is justified by the fact that the inferred amino acid sequences of MOMP among related serovars is relatively conserved (see Figure 20) and the repertoire of *C. trachomatis* gene variants appears to be finite (ref. 16). As may be seen from the data presented in the Examples below, a partially non-reactive immune response was elicited by the MOMP gene of serovar C of *C. trachomatis* to the MOMP gene of serovar MoPn of *C. trachomatis* (Figures 16 to 19).

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis and treatment of chlamydial infections. A further non-limiting discussion of such uses is further presented below.

**1. Vaccine Preparation and Use**

Immunogenic compositions, suitable to be used as vaccines, may be prepared from the MOMP genes or fragments thereof and vectors as disclosed herein. The vaccine elicits an immune response in a subject which includes the production of anti-MOMP antibodies. Immunogenic compositions, including vaccines, containing the nucleic acid may be prepared as injectables, in physiologically-acceptable liquid solutions or emulsions for polynucleotide administration. The nucleic acid may be associated with liposomes, such as lecithin liposomes or other liposomes known in the art, as a nucleic acid liposome (for example, as described in WO 9324640) or the nucleic acid may be associated with an adjuvant, as described in more detail below. Liposomes comprising

cationic lipids interact spontaneously and rapidly with polyanions, such as DNA and RNA, resulting in liposome/nucleic acid complexes that capture up to 100% of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal compartment. Published PCT application WO 94/27435 describes compositions for genetic immunization comprising cationic lipids and polynucleotides. Agents which assist in the cellular uptake of nucleic acid, such as calcium ions, viral proteins and other transfection facilitating agents, may advantageously be used.

Polynucleotide immunogenic preparations may also be formulated as microcapsules, including biodegradable time-release particles. Thus, U.S. Patent 5,151,264 describes a particulate carrier of a phospholipid/glycolipid/polysaccharide nature that has been termed Bio Vecteurs Supra Moléculaires (BVSM). The particulate carriers are intended to transport a variety of molecules having biological activity in one of the layers thereof.

U.S. Patent 5,075,109 describes encapsulation of the antigens trinitrophenylated keyhole limpet hemocyanin and staphylococcal enterotoxin B in 50:50 poly (DL-lactideco-glycolide). Other polymers for encapsulation are suggested, such as poly(glycolide), poly(DL-lactide-co- glycolide), copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(8-hydroxybutyric acid), and polyanhydrides.

Published PCT application WO 91/06282 describes a delivery vehicle comprising a plurality of bioadhesive microspheres and antigens. The microspheres being of starch, gelatin, dextran, collagen or albumin. This

delivery vehicle is particularly intended for the uptake of vaccine across the nasal mucosa. The delivery vehicle may additionally contain an absorption enhancer.

The MOMP gene containing non-replicating vectors may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may include, water, saline, dextrose, glycerol, ethanol, and combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof. Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously, intravenously, intradermally or intramuscularly, possibly following pretreatment of the injection site with a local anesthetic. Alternatively, the immunogenic compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral (intragastric) routes. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkylene glycols or triglycerides. Oral formulations may include normally employed incipients, such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate.

The immunogenic preparations and vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of

the individual's immune system to synthesize the MOMP and antibodies thereto, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgement of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of about 1 μg to about 1 mg of the MOMP gene-containing vectors. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage may also depend on the route of administration and will vary according to the size of the host. A vaccine which protects against only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.

Immunogenicity can be significantly improved if the vectors are co-administered with adjuvants, commonly used as 0.05 to 0.1 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Thus, adjuvants have been

identified that enhance the immune response to antigens. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines.

A wide range of extrinsic adjuvants and other immunomodulating material can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens to produce immune stimulating complexes (ISCOMS), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as Quil A derivatives and components thereof, QS 21, calcium phosphate, calcium hydroxide, zinc hydroxide, an octodecyl ester of an amino acid, ISCOPREP, DC-chol, DDBA and polyphosphazene. Advantageous combinations of adjuvants are described in copending United States Patent Applications Nos.: 08/261,194 filed June 16, 1994 and 08/483,856 filed June 7, 1995, assigned to the assignee hereof and the disclosures of which are incorporated herein by reference thereto (WO 95/34308).

In particular embodiments of the present invention, the non-replicating vector comprising a first nucleotide sequence encoding a MOMP gene of *Chlamydia* may be delivered in conjunction with a targeting molecule to target the vector to selected cells including cells of the immune system.

The non-replicating vector may be delivered to the host by a variety of procedures, for example, Tang et al. (ref. 17) disclosed that introduction of gold microprojectiles coated with DNA encoding bovine growth

hormone (BGH) into the skin of mice resulted in production of anti-BGH antibodies in the mice, while Furth et al. (ref. 18) showed that a jet injector could be used to transfect skin, muscle, fat and mammary tissues of living animals.

**2. Immunoassays**

The MOMP genes, MOMP gene fragments and vectors of the present invention also are useful as immunogens for the generation of anti-MOMP antibodies for use in immunoassays, including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known in the art. In ELISA assays, the non-replicating vector first is administered to a host to generate antibodies specific to the MOMP. These MOMP specific antibodies are immobilized onto a selected surface, for example, a surface capable of binding the antibodies, such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed antibodies, a nonspecific protein, such as a solution of bovine serum albumin (BSA) that is known to be antigenically neutral with regard to the test sample, may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This procedure may include diluting the sample with diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from about 2 to 4 hours, at temperatures such as of the order of about 20° to 37°C.

Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween or a borate buffer. Following formation of specific immunocomplexes between the test sample and the bound MOMP specific antibodies, and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined.

**EXAMPLES**

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

<u>Example 1</u>:

This Example illustrates the preparation of a plasmid vector containing the MOMP gene.

pMOMP expression vector was made as follows. The MOMP gene was amplified from *Chlamydia trachomatis* mouse pneumonitis (MoPn) strain genomic DNA by polymerase chain reaction (PCR) with a 5′ primer (GGGGATCCGCCACCATGCTGCCTGTGGGGAATCCT) (SEQ ID NO: 16) which includes a BamH1 site, a ribosomal binding site, an initiation codon and the N-terminal sequence of the mature MOMP of MoPn and a 3′ primer (GGGGCTCGAGCTATTAACGGAACTGAGC) (SEQ ID NO: 17) which includes the C-terminal sequence of the MoPn MOMP, a XhoI site and a stop codon. The DNA sequence of the MOMP leader peptide gene sequence was excluded. After

digestion with BamH1 and XhoI, the PCR product was cloned into the pcDNA3 eukaryotic II-selectable expression vector (Invitrogen, San Diego) with transcription under control of the human cytomegatovirus major intermediate early enhancer region (CMV promoter). The MOMP gene-encoding plasmid was transferred by electroporation into *E. coli* DH5αF which was grown in LB broth containing 100 μg/ml of ampicillin. The plasmids was extracted by Wizard™ Plus Maxiprep DNA purification system (Promega, Madison). The sequence of the recombinant MOMP gene was verified by PCR direct sequence analysis, as described (ref. 20). Purified plasmid DNA was dissolved in saline at a concentration of 1 mg/ml. The DNA concentration was determined by a DU-62 spectrophotometer (Beckman, Fullerton, CA) at 260 nm and the size of the plasmid was compared with DNA standards in ethidium bromide-stained agarose gel.

The MOMP gene containing so obtained plasmid, pcDNA3/MOMP, and its constitutive elements are shown in Figure 7. A similar plasmid (pM(C)) was constructed from the MOMP gene serovar C of *C. trachomatis*.

Example 2:

This Example illustrates DNA immunization of mice and the results of DTH testing.

A model of murine pneumonia induced by the *C. trachomatis* mouse pneumonitis strain (MoPn) was used (ref. 11). Unlike most strains of *C. trachomatis* which are restricted to producing infection and disease in humans, MoPn is a natural murine pathogen. It has previously been demonstrated that primary infection in this model induces strong protective immunity to reinfection. In addition, clearance of infection is related to CD4 Th1 lymphocyte responses and is dependent on MHC class II antigen presentation (ref. 11).

For experimental design, groups of 4 to 5 week old female Balb/c mice (5 to 13 per group) were immunized intramuscularly (IM) or intranasally (IN) with plasmid DNA containing the coding sequence of the MoPn MOMP gene (1095 bp), prepared as described in Example 1, or with the coding sequence of the *C. trachomatis* serovar $L_2$ CTP synthetase gene (1619 bp (refs. 10, 12), prepared by a procedure analogous described in Example 1. CTP synthetase is a conserved chlamydial cytoplasmic enzyme catalizing the final step in pyrimidine biosynthesis and is not known to induce protective immunity. Negative control animals were injected with saline or with the plasmid vector lacking an inserted chlamydial gene.

For IM immunization, both quardiceps were injected with 100 μg DNA in 100 μl of saline per injection site on three occasions at 0, 3 and 6 weeks. For IN immunization, anaesthetized mice aspirated 25 μl of saline containing 50 μg DNA on three occasions at 0, 3 and 6 weeks. As a positive control, a separate group of mice received 5 x $10^6$ inclusion forming units (IFUs) of MoPn EBs administered intraperitoneally in incomplete Freund's adjuvant according to the above schedule. At week 8, all groups of mice had sera collected for measuring antibodies and were tested for delayed-type hypersensitivity (DTH) to MoPn Ebs by footpad injection (ref. 13).

A positive 48 and 72 hour DTH reaction was detected among mice immunized with MOMP DNA or with MoPn Ebs but not among mice immunized with the blank vector (see Figure 1). The DTH reaction elicited with MOMP DNA delivered intranasally was comparable to that observed among mice immunized with EBs. No DTH reaction was detected among the groups of mice vaccinated with CTP synthetase DNA (see Table 1 below). Thus, injection of

MOMP DNA generated a DTH reaction that was capable of recall by naturally processed peptides from *C. trachomatis* EBs while injection of CTP synthetase DNA failed to do so.

Example 3:

This Example illustrates DNA immunization of mice and the generation of antibodies.

Injection of CTP synthetase DNA as described in Example 2 resulted in the production of serum antibodies to recombinant CTP synthetase (Table 1) (ref. 14). Antigen-specific serum Abs were measured by ELISA. Flat-bottom 96-well plates (Corning 25805, Corning Science Products, Corning, NY) were coated with either recombinant chlamydial CTP-synthetase (1 μg/ml) or purified MoPn EBs (6 x $10^4$ IFU/well) overnight at 4°C. The Plates were rinsed with distilled water and blocked with 4% BSA PBS-Tween and 1% low fat skim milk for 2 hours at room temperature. Dilutions of sera samples were performed in 96-well round bottom plates immediately prior to application on the antigen coated plates. The plates were incubated overnight at 4°C and washed ten times. Biotinylated goat anti-mouse IgG1 or goat anti-mouse IgG2a (Southern Biotechnology Associates, Inc. Birmingham, AL) were next applied for 1 hour at 37°C. After washing; streptoavidin-alkaline phosphatase conjugate (Jackson ImmunoResearch Laboratories, Inc. Mississagua, Ontario, Canada) were added and incubated at 37°C for 30 min. Following another wash step, phosphatase substrate in phosphatase buffer (pH 9.8) was added and allowed to develop for 1 hour. The plates were read at 405 nm on a BIORAD 3550 microplate reader.

IgG2a antibody titers were approximately 10-fold higher than lgG1 antibody titers suggesting that DNA

immunization elicited a more dominant $T_{H1}$-like response. Injection of MOMP DNA as described in Example 2 resulted in the production of serum antibodies to MOMP (Table 2) as detected in an immunoblot assay (Figure 2). However, neither CTP synthetase DNA nor MOMP DNA immunized mice produced antibodies that bound to native *C. trachomatis* EBs (Table 1), suggesting that the antibody responses may not to be the dominantly protective mechanism. A comparison of serum lgG subclasses, lgG2a (Panels A and C and $lgG_1$ (Panels B and D) against MOMP protein (Panels A and B) or MoPn (Panels C and D) induced by DNA immunization as described above, is contained in Figure 4.

Example 4:

This Example illustrates DNA immunization of mice to achieve protection.

To investigate whether a cell-mediated immune response elicited by MOMP DNA was functionally significant, *in vivo* protective efficacy was evaluated in mice challenged intranasally with 1 x $10^3$ IFU of *C. trachomatis* MoPn. To provide a measure of Chlamydia-induced morbidity, the loss in body weight was measured over 10 days following challenge with *C. trachomatis* (see Figure 2, Panel A). Mice injected with the unmodified vector were used as negative controls and mice immunized with EBs were used as positive controls. Mice immunized with MOMP DNA intranasally maintained a body weight comparable to that observed among EB immunized mice. Mice intramuscularly immunized with MOMP DNA lost body mass but did so at a rate less than the negative control group.

A more direct measure of the effectiveness of DNA vaccination is the ability of mice immunized with MOMP DNA to limit the *in vivo* growth of Chlamydia following a sublethal lung infection. Day 10 post-challenge is the

time of peak growth (ref. 13) and was chosen for comparison of lung titers among the various groups of mice. Mice intranasally immunized with MOMP DNA had chlamydial lung titers that were over 1000-fold lower ($log_{10}$ IFU 1.3±0.3; mean ± SEM) than those of control mice immunized with the blank vector ($log_{10}$ IFU 5.0±0.3; $p<0.01$) (see Figure 2, Panel B). Mice intramuscularly immunized with MOMP DNA had chlamydial lung titers that were more than 10-fold lower than the unmodified vector group ($p = 0.01$). Mice intranasally immunized with MOMP DNA had significantly lower chlamydial lung titers than mice immunized with MOMP DNA intramuscularly ($log_{10}$ IFU 1.3±0.8 versus $log_{10}$ IFU 0.66±0.3 respectively; $p = 0.38$). The substantial difference (2.4 logs) in chlamydial lung titers observed between the intranasally and intramuscularly MOMP DNA immunized mice suggests that mucosal immunization is more efficient at inducing immune responses to accelerate chlamydial clearance in the lung. The lack of protective effect with the unmodified vector control confirms that DNA *per se* was not responsible for the immune response. Moreover, the absence of protective immunity following immunization with CTP synthetase DNA confirms that the immunity was specific to the MOMP DNA (see Table 1). Figure 5 shows similar challenge data at a higher challenge dose.

Example 5:

This Example describes the construction of p½MOMP.

A PCR cloned MoPn gene was constructed containing a deletion mutation in codon 177. This mutation yields a truncated MOMP protein containing approximately 183 amino-terminal amino acids (ref. 10). This construct, termed p½MOMP, was cloned into the vector pcDNA3 (Invitrogen), in the manner described in Example 1 for the full length MOMP gene.

In addition, a series of vectors was generated containing fragments of the nucleotide sequence of the MoPn MOMP gene by PCR cloning and subsequent cloning into the vector pcDNA3 to generate plasmids pCV1, pCV2, pCV3, pCV4 and pCV5, respectively containing the portions of the MoPn MOMP gene shown in Figure 8.

<u>Example 6</u>:

This Example illustrates immunization of mice with p½MOMP, pCV1, pCV2, pCV3, pCV4 and pCV5.

Balb/c mice were immunized in the quadriceps three times at a three week intervals with 100 μg of p½MOMP, pCV1, pCV2, pCV3, pCV4 and pCV5 DNA.

Fifteen days after the last immunization and 60 days after the first injection, mice were bled for measurement of serum antibodies of MoPn EBs in an EIA assay and were injected in the footpad with 25 μl ($5 \times 10^4$ inclusion forming units) of heat killed EBs for measurement of DTH which was measured at 72 hours (ref. 13). Mice were intranasally challenged with 1000 infectious units of MoPn and their body weight measured daily for the subsequent 10 days. At that time, mice were sacrificed and quantitative cultures of MoPn in the lung determined (ref. 13).

Table 3 shows that p½MOMP immunization elicited a positive DTH response to footpad injection of MoPn EBs. Low titers (approximate titer 1/100) serum antibodies to surface determinants on EBs were also detected at day 60 post vaccination. Immunization with the unmodified vector elicited neither serum antibodies nor a DTH response. Figure 11 shows that immunization with pCV1, pCV2, pCV3, pCV4 and pCV5 elicited variable positive DTH responses to footpad injection of MoPn EBs. pCV3 and pCD5 elicited greater responses comparable to pMOMP.

Figure 6, Panel A shows that p½MOMP immunization evoked a protective immune response to MoPn challenge as measured by change in body weight post infection and by the *in vivo* growth of MoPn in lung tissue day 10 post challenge. The *in vivo* growth among saline treated mice was $\log_{10}$ 5.8 ± 0.21 and among p½MOMP immunized mice was $\log_{10}$ 3.9 ± 0.25, p<.001, Figure 2, Panel B. As a positive control, mice immunized with heat killed MoPn EBs or recovered from prior infection with MoPn were markedly and equivalently protected against challenged infection (p<.0001).

Figure 9 shows that pCV2, pCV3 and pCD5 immunization evoked a protective immune response to MoPn challenge as measured by loss in body weight post infection comparable to that in mice protected against disease, as seen by lung titres. However, the specific domains eliciting these immune responses do not include those predicted in the art to contain T-cell epitopes. In this regard, several groups have attempted to define MOMP T-cell epitopes (refs. 22 to 26). All of those studies used overlapping synthetic peptides to various regions of the MOMP protein to prime mice. None of the predicted epitopes fall within regions that have been found to be protective.

As may be seen in this Example, using a frame-shift deletion mutant at codon 177 of the MOMP gene, significant protective immunity to challenge infection was elicited suggesting that protective sites can be found in the amino terminal half of the protein. In addition, it has further shown in this Example that the vectors containing specific segments of the MOMP gene were able to protect against disease, based on body weight loss, namely pCV2 and pCD5. In addition, vectors

pCV3 and pCD5 were able to protect against infection, based on lung titres.

Example 7:

This Example illustrates the effect of DNA immunization of mice with pM(C).

The pcDNA3 vector containing the MOMP gene for serovar C of *C. trachomatis*, prepared as described in Example 1, was immunized into mice following the procedure of Example 2 and various results charted graphically in comparison to the results obtained using pMOMP from MoPn strain.

In this regard, Ig2a antibody responses (Figure 16), footpad swelling responses (Figure 17), proliferation of splenocytes (Figure 18) and IFN-γ secretion (Figure 19) were determined following the procedures of Example 3, Example 2 and Example 6 respectively.

## SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides a method of nucleic acid, including DNA, immunization of a host, including humans, against disease caused by infection by a strain of *Chlamydia*, specifically *C. trachomatis*, employing a non-replicating vector, specifically a plasmid vector, containing a nucleotide sequence encoding a major outer membrane protein (MOMP) of a strain of *Chlamydia* or a fragment of MOMP which generates a MOMP-specific immune response and a promoter to effect expression of MOMP in the host. Modifications are possible within the scope of this invention.

**Table 1**

Serum antibody titers and delayed-type hypersensitivity (DTH) responses and *in vivo* growth of *Chlamydia trachomatis* following pCTP synthetase or MoPn EB immunization. Results are presented as means ± SEM.

| | Anti-MoPn EB antibodies ($\log_{10}$) | | anti-rCTP synthetase antibodies ($\log_{10}$) | | Anti-EB DTH (mm x $10^2$) | $\log_{10}$ IFU/lung d10 post challenge |
|---|---|---|---|---|---|---|
| | IgG1 | IgG2a | IgG1 | IgG2a | | |
| Saline (n = 9) | <2 | <2 | <2 | <2 | 4.5 ± 1.5 | 4.9 ± 2.4 |
| pCTP synthetase (n = 11) | <2 | <2 | 3.8 ± .3 | 4.7 ± .1 | 1.4 ± 1.5 | 4.7 ± .13 |
| EB (n = 4) | 5.0 ± .3 | 4.8 ± .3 | 3.6 ± .8 | 2.9 ± 0 | 15.2 ± 2.0 | 0 |

**Table 2**

Serum antibody Elisa titers to *Chlamydia trachomatis* mouse pneumonitis recombinant MOMP and EBs were measured 60 days after the initial immunization among mice immunized with blank vector alone (pcDNA3), vector containing the MOMP gene (pMOMP) and vector containing the CTP synthetase gene (pCTP). Non-immunized mice were also tested.

| | rMOMP | | EB | |
|---|---|---|---|---|
| Immunogen | IgG2a | IgG1 | IgG2a | IgG1 |
| pcDNA3 | <2.6* | <2.6 | <2.6 | <2.6 |
| pMOMP | 3.77 ± 0.1 | 2.90 ± 0.14 | 3.35 ± 0.11 | <2.6 |
| pCTP | ND | ND | <2.6 | <2.6 |
| Preimmunization | <2.6 | <2.6 | <2.6 | <2.6 |

* $\log_{10}$ mean ± SE IgG isotype speciic antibody titer

ND = not done

**Table 3**

Immune responses at day 60 following p½MOMP, EB or blank vector (pcDNA3) immunization of mice.

| Immunogen | EB $IgG_{2a}$ antibody titer ($\log_{10}$) | DTH response to EB (mm x $10^2$) |
|---|---|---|
| EB (n = 13) | 5.6 ± 0.4 | 9.6 ± 2.0 |
| p½MOMP (n = 13) | 2.0 ± 0 | 6 ± 1.6 |
| pcDNA3 (n = 13) | 1.3 ± 0 | 1 ± 1 |

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